



Islands in the stream: Distribution of *Myotis attenboroughi* (Chiroptera, Vespertilionidae) in Grenada and mainland South America illuminates the evolutionary history of Caribbean *Myotis*

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Abstract

Myotis attenboroughi was recently described based on specimens from Tobago Island and considered an endemic species until now. Based on morphological and molecular data, we extended its occurrence to Grenada and Suriname. The presence of *M. attenboroughi* in Grenada, Tobago and mainland South America can be explained by the intermittent connections between these islands and the continent during the Plio-Pleistocene glaciations. However, our analyses recovered an *Myotis* individual from Grenada phylogenetically related to *Myotis nyctor* from Barbados, although it is morphologically like the other specimens of *M. attenboroughi*, revealing a surprising incongruence between genotype and phenotype. Barbados is a geologically recent island and historically unconnected with other Caribbean islands or with South America. We hypothesized that the invasion of the *M. nyctor* lineage from Barbados to Grenada was the result of an extreme overwater dispersal, perhaps driven by wind streams during tropical storms. Subsequently, introgression of lineages may have occurred through hybridization, which can explain the discordance between the phenotype (like *M. attenboroughi*) and the genotype (like *M. nyctor*) of this specimen. Additional comments on the formation of the Caribbean *Myotis* assemblage are presented from the perspective of new evolutionary discoveries for this genus.

Key Words

Barbados, Caribbean biogeography, historical DNA, *Myotis nyctor*, Suriname, taxonomy

Introduction

Myotis has a worldwide distribution, occurring in every region of the globe except for parts of Australasia and the polar regions and is the most diverse genus within the order Chiroptera, comprising 139 valid species (Moratelli et al. 2019; Simmons and Cirranello 2024). Thirty-six neotropical species are currently recognized (Novaes et al. 2024), seven of which are found on the Caribbean islands (Novaes et al. 2021), being: *M. nesopolus* Miller, 1900

in Bonaire and Curaçao; *M. dominicensis* Miller, 1902 in Dominica and Guadeloupe; *M. martiniquensis* LaVal, 1973 in Martinique; *M. nyctor* LaVal & Schwartz, 1974 in Barbados and Grenada; *M. pilosatibialis* LaVal, 1973 (possibly *M. armiensis* Carrión-Bonilla & Cook, 2021), *M. riparius* Handley, 1960 in Trinidad; and *M. attenboroughi* Moratelli et al., 2017 in Tobago (LaVal 1973; Larsen et al. 2012; Moratelli et al. 2017; Novaes et al. 2021).

Miller and Allen (1928) recognized only *M. nigricans* (Schinz, 1821), with three subspecies in the Caribbean

islands. Populations from Grenada and Trinidad and Tobago were assigned to the nominative subspecies, those from Dominica to *M. nigricans dominicensis* Miller, 1902; and those from Curaçao to *M. nigricans nesopolus* Miller, 1900. Later, LaVal (1973) elevated *dominicensis* to the species level and described *M. martiniquensis* from Martinique and Barbados. LaVal and Schwartz (1974) subsequently recognized Barbados' populations as distinct and described a new subspecies, *M. martiniquensis nyctor*. Genoways and Williams (1979) elevated *nesopolus* to the species level and reported its occurrence in Bonaire, while Genoways et al. (1998) reported *M. nigricans* from Grenada. Masson and Breuil (1992) reported the occurrence of one *Myotis* species from Guadeloupe, presumably assigned to *dominicensis*. Larsen et al. (2012) raised *M. martiniquensis nyctor* to the species level, noting its presence in Barbados, with pending records from Grenada based on three museum specimens that were not examined by the authors. Moratelli et al. (2017) examined these specimens, assigning USNM 254717 to *Peropteryx* (Emballonuridae), and CM 83427 and USNM 252600 to *M. nyctor*. In addition, these authors revised the population of *Myotis nigricans* from Tobago and, based on morphological and molecular evidence, assigned this population to a new species, *Myotis attenboroughi* (see Moratelli et al. 2017). Recently, Novaes et al. (2021) reviewed the Venezuelan and Caribbean *Myotis*, suggesting that populations from Barbados and Grenada may represent distinct taxa, and emphasized the need for additional morphological and molecular data to support this hypothesis.

Based on novel molecular and morphological evidence, we extend the occurrence of *M. attenboroughi* to Grenada (based on the reexamination of the specimen USNM 252600, collected in 1938 and previously assigned to *M. nyctor*) and to mainland South America (based on the specimen CM 77705 previously assigned to *Myotis* cf. *nigricans*). Our reassessment of the taxonomic identity of these and other historical specimens from Tobago (i.e., USNM 540692) and Grenada (i.e., CM 83427) also allowed us to further discuss the evolution of *Myotis* in the Caribbean.

Material and methods

Our research group conducted a comprehensive review of neotropical *Myotis* by analyzing over 7,500 specimens and hundreds of DNA sequences. This extensive analysis resulted in the recognition of 11 new species and several other nomenclatural acts (e.g., Moratelli et al. 2011, 2013; Novaes et al. 2022a, b). The present study was based on the analysis of datasets comprising molecular, morphometric, and discrete morphological characters. We adopted the Phylogenetic Species Concept (Wheeler 1999), considering monophyly and diagnosability as criteria for species recognition (Gutiérrez and Garbino 2018).

Molecular data and analyses

Molecular analyses were based on 122 sequences of the mitochondrial cytochrome b gene (cytb, ca. 1,140 bp) from New World *Myotis* species and three outgroups (Appendix 1). Most sequences (123 out of 125, including outgroups) were obtained from NCBI's GenBank, including the sequences from CM 77705 (Suriname) and CM 83427 (Grenada). The remaining two sequences were generated in this study. Tissue samples of *Myotis* from Grenada (USNM 252600) and Tobago (USNM 540692) were obtained from toe clips from historical specimens deposited in the Smithsonian National Museum of Natural History, Washington D.C., USA, following rigorous sampling procedures described in Abreu et al. (2020). DNA extractions were performed in an isolated historical DNA facility at the Smithsonian Center for Conservation Genomics (CCG), using a standard phenol-chloroform protocol (McDonough et al. 2018), including a long (48–72 hours) lysis step. We did not perform specific amplifications for the cytb gene. Fragments of mtDNA were obtained as a byproduct (off-target sequences) of the capture and enrichment of Ultraconserved Elements sequenced for a parallel study conducted by our team. Sequencing was performed on Illumina Hi-Seq 4000 150 PE at the Vincent J. Coates Genomics Sequencing Laboratory at the University of California, Berkeley. A detailed description of the procedures used in the preparation of genomic libraries, DNA quantification, and sequencing is available in Abreu et al. (2020). To obtain the cytb gene sequences, we mapped the clean Illumina reads against a reference mitochondrial genome from GenBank, using the “Map to Reference” tool in Geneious R11 (Kearse et al. 2012). Mapping and sequence assembly parameters followed Abreu et al. (2020).

The cytb dataset was aligned using the UPGMA clustering method implemented in the MUSCLE algorithm (Edgar 2004) in the MEGA X software (Kumar et al. 2018) with default settings. The evolutionary model of nucleotide substitution was chosen for phylogenetic analyses using the software JModelTest 2 (Darriba et al. 2012), employing the Bayesian Information Criterion (BIC). The Hasegawa–Kishino–Yano model (Hasegawa et al. 1985) yielded the best fit to our dataset regarding the substitution of nucleotides, corrected for rate heterogeneity with gamma distribution and proportion of invariant sites parameters (i.e., HKY + Γ + I). Phylogenetic reconstruction was performed using the Bayesian Inference (BI) probabilistic method (Huelsenbeck et al. 2001) in the software MrBayes v. 3.4 (Ronquist and Huelsenbeck 2003) using the coupled Markov Chain Monte Carlo (MCMC). Four simultaneous Markov chains were performed for 100,000,000 generations with trees sampled every 10,000 generations. The first 26,000 trees were discarded as burn-in. Posterior probabilities were calculated from the consensus of the remaining trees. The confidence of the Bayesian sampling was verified for the free parameters using the effective sample size (ESS) statistic implemented in the

software Tracer v. 1.5 (Rambaut and Drummond 2009). Convergence was checked by plotting log-likelihood values against the generation time for each model, with all parameters showing ESS greater than 300 and asymptotically convergence indicating reliable performance.

Pairwise genetic distances within and among *Myotis* species were estimated using the HKY model implemented in ‘ape 5.0’ package for R software (Paradis and Schliep 2019), which measures the distance between pairs of sequences by estimating the proportion of different nucleotides between them.

Morphological data and analyses

For the morphological analyses and comparisons, we examined 14 specimens of *M. attenboroughi* (13 from Tobago, including holotype and paratypes; and one from Grenada); eight of *M. nyctor* from Barbados (including a paratypes); and one specimen from Grenada tentatively identified as *M. cf. nyctor* (Appendix 2). Quantitative morphological data were based on 16 skull dimensions, representing different axes of the length and width of the skull, rostrum, and mandible, and three external measurements (Table 1). Measurements were taken using digital calipers accurate to 0.01 mm, exclusively from individuals classified as adults based on closed epiphyses (see Brunet-Rossini and Wilkinson 2009). Additionally, measurements of total length, tail length, hindfoot length and body weight were recorded from the specimen’s tag and used for comparative purposes only.

Principal Component Analysis (PCA) was used to identify general trends in variation in size and shape vari-

ation of the skull between forms of Barbados, Grenada, and Tobago. This analysis was performed in R using the MASS (Vanables and Ripley 2002) and Lattice packages (Sarkar 2008). As PCA requires a complete dataset without missing data, cranial measurements that could not be taken from the specimen due to skull fractures and losses of parts were estimated from the log-transformed dataset using the EM algorithm implemented in the R package Amelia II (Honaker et al. 2011).

Qualitative morphological analyses were based on six cranial and external characters traditionally used in Neotropical *Myotis* taxonomy (q.v., LaVal 1973; Moratelli et al. 2013; Novaes et al. 2022a). Fur color was also used to describe the variation, following the nomenclature used in the color catalog of Ridgway (1912).

Results

Phylogenetic inference and genetic distances

The specimen of *Myotis* from Grenada (USNM 252600) was recovered within the *M. attenboroughi* clade, which also includes the paratype USNM 540692 (Fig. 1). The specimen from Paramaribo, Suriname (CM 77705), was recovered as sister to all Caribbean samples (Tobago and Grenada) of *M. attenboroughi*. The specimen CM 83427 from Grenada was recovered within a clade composed by specimens of *M. nyctor* from Barbados, and here it is being treated as *M. cf. nyctor*. Both clades corresponding to *M. attenboroughi* and *M. nyctor* are closely related to other *Myotis* species from the Caribbean and northern South America, all included in the *albescens* species group (Fig. 1).

Table 1. Skull dimensions used to perform morphological comparisons in Neotropical *Myotis* species. Measurements were taken in millimeters.

Measurement	Description
Forearm Length (FL)	From the elbow to the distal end of the forearm including carpals
Third metacarpal length (3ML)	From the distal end of the forearm including carpals to the distal end of the 3 rd metacarpal.
Lenght of dorsal fur (LDF)	Length of the longest hairs at the midpoint of the scapulae
Length of ventral fur (LVF)	Length of the longest hairs at the midpoint of the sternum
Mandibular length (MAL)	From the mandibular symphysis to the condyloid process
Mandibular toothrow length (MAN)	From the lower canine to third molar
Greatest length of skull (GLS)	From the apex of upper internal incisors to the occiput
Condylo-canine length (CCL)	From the anterior surface of upper canines to a line connecting the occipital condyles
Condylo-incisive length (CIL)	From the apex of upper internal incisors to a line connecting the occipital condyles
Condylo-basal length (CBL)	From the anterior region of premaxilla to a line connecting the occipital condyles
Basal length (BAL)	Least distance from the apex of upper internal incisors to the anterior margin of the foramen magnum
Zygomatic breadth (ZYG)	Greatest breadth across the outer margins of the zygomatic arches
Mastoid breadth (MAB)	Greatest breadth across the mastoid region
Braincase breadth (BCB)	Greatest breadth of the globular part of the braincase
Interorbital breadth (IOB)	Least breadth between the orbits.
Postorbital constriction (POB)	Least breadth across frontals posterior to the postorbital bulges
Breadth across canines (BAC)	Greatest breadth across outer edges of the crowns of upper canines including cingulae
Breadth across molars (BAM)	Greatest breadth across outer edges of the crowns of upper molars
Maxillary toothrow length (MTL)	From the upper canine to third molar
Upper molar toothrow length (M1M3)	From M1 to M3

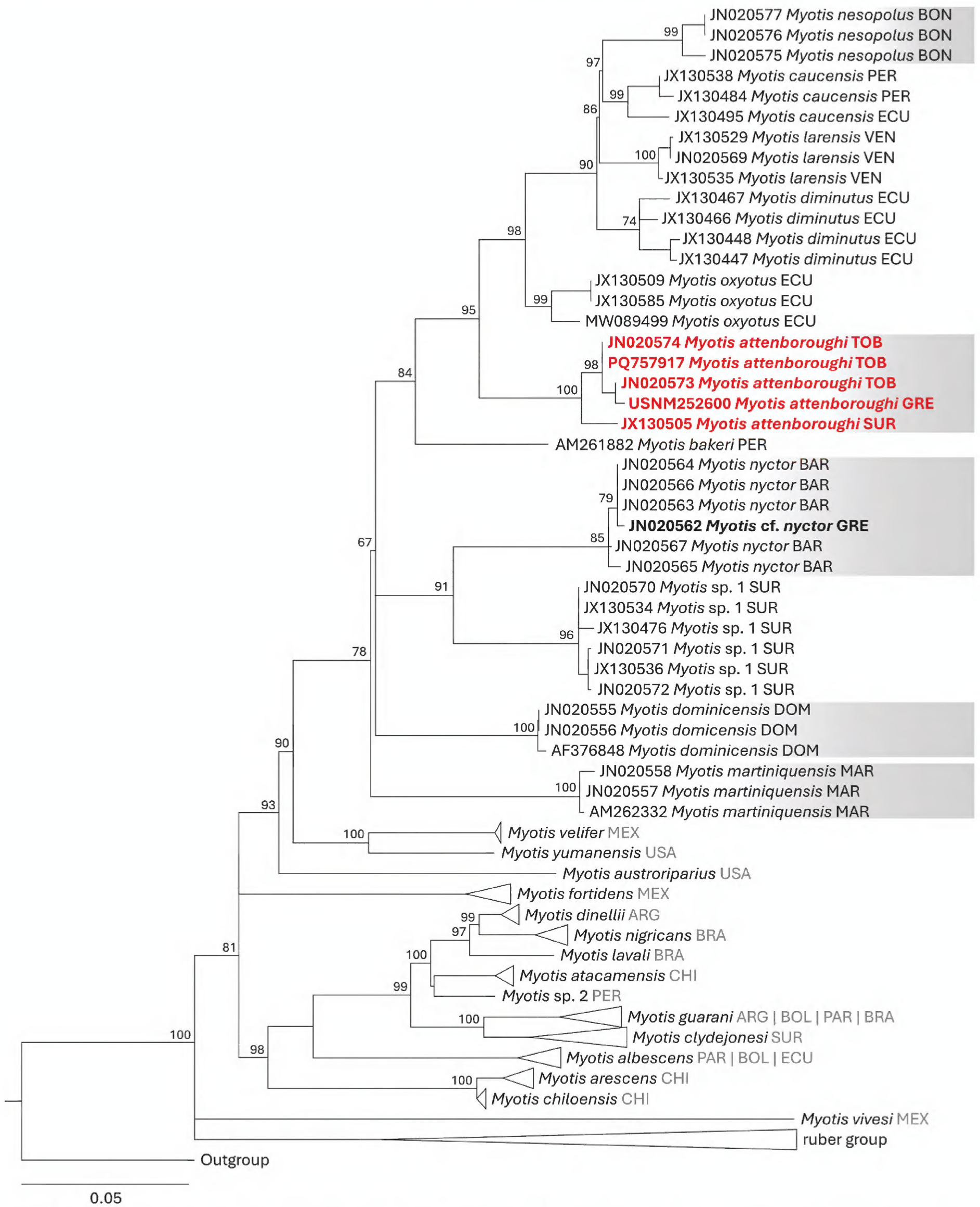


Figure 1. Phylogenetic tree based on Bayesian Inference of cytochrome-b sequences of species of Neotropical *Myotis*. Nodal support was calculated by posterior probabilities. Caribbean species are highlighted by gray bars.

The estimated HKY genetic distances indicated that *M. attenboroughi* from Suriname (CM 77705) diverges by approximately 1.5% from samples from Tobago and Grenada. The average divergence within the clade composed of Tobago and Grenada samples of *M. attenboroughi* was less than 0.1%. Genetic distances among *M.*

attenboroughi and phylogenetic closely species range from 6 to 8% (Table 2). *Myotis attenboroughi* from Grenada (USNM 252600) diverges by about 10% from *M. nyctor* from Barbados, alike *M. attenboroughi* from Grenada diverges in about 10% from *M. cf. nyctor* from Grenada (CM 83427).

Table 2. Average Hasegawa–Kishino–Yano genetic distances within (boldface along diagonal) and among (below diagonal) *Myotis* species based on cytochrome-b gene sequences.

Species	1	2	3	4	5	6	7
1. <i>M. attenboroughi</i>	[0.008]						
2. <i>M. larensis</i>	0.073	[0.006]					
3. <i>M. nesopolus</i>	0.080	0.051	[0.002]				
4. <i>M. diminutus</i>	0.069	0.020	0.044	[0.009]			
5. <i>M. caucensis</i>	0.090	0.041	0.040	0.037	[0.031]		
6. <i>M. oxyotus</i>	0.059	0.063	0.072	0.044	0.069	[0.015]	
7. <i>M. nyctor</i>	0.111	0.132	0.145	0.116	0.137	0.124	[0.006]

Morphological description and comparisons

The specimen of *M. attenboroughi* from Grenada (USNM 252600) is an adult male preserved as dry skin and skull (Figs 2, 3). It is a small-sized specimen (forearm length 32.6 mm; other measurements in Table 3), with medium-sized ears (14 mm). The fur is silky in texture and medium-sized (LDH 5.6 mm, LVH 4.5 mm). The dorsal fur is bicolor, with dark brown bases (ca. 1/2 of the total length of the fur), and Mummy Brown tips (ca. 1/2), without a defined contrast between the bands. Ventral fur is strongly bicolor, with blackish bases and Light-Buff tips (Fig. 2). Membranes are Mummy Brown. Uropatagium is attached to the foot by a broad band of membrane. Dorsal surfaces of elbow and tibia are naked. The uropatagium lacks the fringe of hairs along the trailing edge.

Like in other neotropical *Myotis*, the dental formula is 2/3, 1/1, 3/3, 3/3 = 38. The skull is comparatively small, lacking a sagittal crest; lambdoidal crests are present, but very low; parietals are slightly inclined (Fig. 3). The occipital region is rounded, projecting behind the posterior surfaces of occipital condyles. The second upper premolar (P3) is aligned in the toothrow, smaller than P2 and P4, and visible in labial view. The set of qualitative morphological characters of the Grenada specimen (USNM 252600) is in accordance with the holotype of *M. attenboroughi* from Tobago (USNM 540693) and with the diagnosis presented in the original description (i.e., Moratelli et al. 2017). The morphometric measurements of this specimen are within the known range for *M. attenboroughi* from Tobago (including holotype and paratypes; Table 3).

Myotis attenboroughi and *M. nyctor* are phenotypically quite similar but they can be distinguished by a set of morphological characters (Figs 4, 5). *Myotis nyctor* has silky, medium-sized fur (LDH 6.3 mm, LVH 4.5 mm). Dorsal fur bicolored, with Cinnamon Brown to Mummy Brown tips (ca. 1/2 of the total length of the fur) and darker bases, without well-marked limits between bands. Ventral fur strongly bicolored, with dark brown bases and Light-Buff tips. *Myotis nyctor* differs from *M. attenboroughi* in general size (Table 3) and craniodental characters, which include its longer and narrow rostrum; larger canines; less globose braincase; and narrower interorbital constriction.

The specimen from Grenada (CM 83427), phylogenetically grouped with *M. nyctor* samples from Barbados,

presented external and skull morphology virtually identical to *M. attenboroughi* specimen (USNM 252600) also from Grenada. The specimen CM 83427 is an adult male preserved as dry skin and skull. Despite exhibiting no discrete distinguishable character, these two individuals from Grenada (CM 83427 and USNM 252600) were also recovered closely positioned within the morphospace based on the PCA analysis. Both specimens presented skull dimensions similar to *M. attenboroughi*, being recovered close to the cluster formed by samples of *M. attenboroughi* from Tobago (Fig. 6). In this analysis, the first principal component (PC1) accounted for almost 98% of the variation, which was mainly driven by the variation found in measurements associated with condylo-incisive length, condylo-basal length, greatest length of skull, and condylo-canine length (Table 4).

Discussion

Our results unequivocally supported the identification of the specimen USNM 252600 from Grenada as *M. attenboroughi*. This specimen was captured by S. Gates in March 1938 and originally identified as *M. nigricans*. Genoways et al. (1998) examined this specimen and collected an additional individual during an expedition to Grenada in June 1986 (CM 83427), confirming their identity as *M. nigricans* and arguing that the specimens from Grenada are very similar to *M. nigricans* from mainland Venezuela. Later, Larsen et al. (2012) sequenced the cytb gene from the specimen CM 83427 and found this specimen within a clade composed of *M. nyctor* from Barbados. However, Larsen et al. (2012) noted that cranial and external measurements of specimens from Grenada were outside the range observed for *M. nyctor* from Barbados, which corroborated the Genoways et al. (1998) observations. Based on qualitative morphological analyses, Moratelli et al. (2017) supported the results of Larsen et al. (2012) and reidentified the specimens USNM 252600 and CM 83427 as *M. nyctor*, thus rejecting the hypothesis of *M. nigricans* occurring in Grenada. In a critical review of Caribbean *Myotis*, Novaes et al. (2021) suggested that the taxonomic status of populations from Grenada still needed to be assessed, considering the presence of morphological disparities and possible retention of ancestral polymorphism resulting from recent speciation.



Figure 2. Dorsal (left) and ventral (right) view of the specimen of *Myotis attenboroughi* (USNM 252600) from Grenada Island.

Table 3. Selected measurements of *Myotis attenboroughi* and *M. nyctor*. Descriptive statistics include the mean, range (in parentheses), and sample size. Measurements are rounded to the nearest 0.1 mm. See Table 1 for abbreviations.

Measurements	<i>M. attenboroughi</i> Grenada USNM 252600	<i>M. attenboroughi</i> Tobago	<i>M. cf. nyctor</i> Grenada CM 83427	<i>M. nyctor</i> Barbados
FL	32.6	32.3 (31.4–33.3) 5	33.5	35.3 (34.6–35.9) 7
3ML	30.1	29.5 (28.5–30.3) 4	–	32.2 (31.4–32.7) 7
LDF	5.6	7.0 (6.0–8.0) 4	5.9	6.2–6.5 (2)
LVF	4.5	5.0 (5.0–6.0) 4	3.7	4.0–5.0 (2)
MAL	9.2	9.2 (8.9–9.5) 10	9.1	10.4 (10.2–10.6) 7
MAN	5.1	5.2 (5.1–5.3) 10	5.1	5.9 (5.4–6.0) 7
GLS	–	12.9 (12.5–13.1) 10	13.2	14.3 (14.1–14.4) 7
CCL	11.6	11.5 (11.1–11.7) 10	11.7	12.8 (12.6–13.1) 7
CBL	–	12.1 (11.8–12.4) 10	12.2	13.5 (13.3–13.7) 7
CIL	–	12.3 (12.0–12.6) 10	12.4	13.8 (13.4–14.0) 7
BAL	–	11.0 (10.8–11.4) 10	11.2	12.5 (12.1–12.7) 7
ZYG	8.2	7.9 (7.8–8.1) 6	8.3	(8.1–8.3) 2
MAB	6.7	6.5 (6.4–6.7) 10	6.7	7.2 (6.9–7.3) 7
BCB	6.2	6.0 (5.9–6.2) 10	6.2	6.6 (6.4–6.7) 7
IOB	4.2	4.2 (4.1–4.3) 10	4.0	4.3 (4.0–4.6) 7
POB	3.6	3.2 (3.2–3.5) 10	3.4	3.3 (3.3–3.4) 7
BAC	3.1	3.1 (2.9–3.3) 10	3.2	3.6 (3.6–3.8) 7
BAM	5.0	5.2 (5.1–5.3) 10	5.2	5.4 (5.4–5.5) 7
MTL	4.8	4.9 (4.8–5.0) 10	4.8	5.6 (5.4–5.7) 7
M1M3	2.7	2.8 (2.7–2.8) 10	2.7	3.0 (2.9–3.0) 7



Figure 3. Skull profiles of *Myotis attenboroughi* (USNM 252600) from Grenada Island in dorsal, ventral, and lateral views.

The present record of *M. attenboroughi* on the island of Grenada extends the geographic range of this species to two localities in the Caribbean islands (Grenada and Tobago) and one locality in the mainland South America (Suriname; Fig. 7). It is likely that this species has an even wider distribution in northern South America and may oc-

cur in the lowlands along coastal rainforests of the Guiana Shield and northeastern Venezuela, which corroborates the observations made by Genoways et al. (1998).

The specimen CM 83427 morphologically matches with the diagnosis of *M. attenboroughi* (see Moratelli et al. 2017), being virtually identical to USNM 252600 from Grenada.



Figure 4. Skins of the paratype of *Myotis nyctor* (KU 109473) from Barbados (A, B) and holotype of *Myotis attenboroughi* (USNM 540693) from Tobago (C, D).

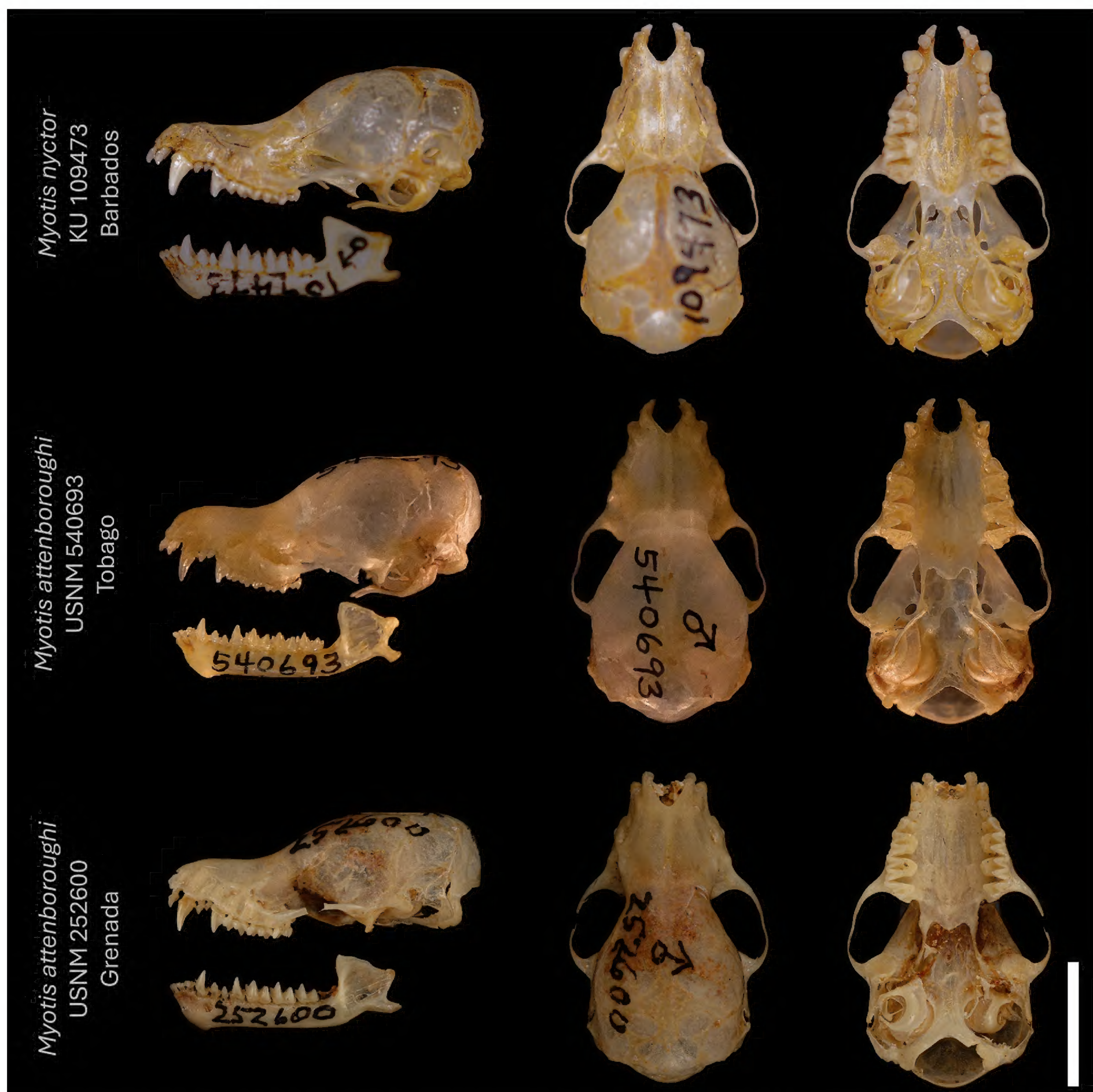


Figure 5. Skulls of the paratype of *Myotis nyctor* (KU 109473) from Barbados, holotype of *Myotis attenboroughi* (USNM 540693) from Tobago, and a specimen of *Myotis attenboroughi* (USNM 252600) from Grenada.

However, our phylogenetic inference recovered this specimen as belonging to the *M. nyctor* clade from Barbados, as previously shown by Larsen et al. (2012), revealing a surprising incongruence between genotype and phenotype. One could argue that the discrepancy between morphological and molecular analyses may be due to issues related with the tissue sampling for DNA analysis and/or with the lab routine for molecular data generation. Although this hypothesis cannot be completely ruled out without the re-sequencing these specimens, at least we can attest to the best practices while working with historical DNA for the individual USNM 252600. Sampling followed rigorous procedures described and tested in McDonough et al. (2018) and Abreu et al. (2020), and data generation (DNA extraction and genomic library preparation) took place in state-of-the-

art facilities with protocols and equipment exclusively used for ancient and historical samples. Moreover, for specimen USNM 252600 we also generated thousands of nuclear ultraconserved elements (data not shown), and these data also corroborate the phylogenetic placement found here for this specimen. Therefore, if data generation issues were ruled out, this unexpected result should be explained in light of the complex evolutionary history of Caribbean *Myotis*.

The Caribbean *Myotis* assemblage originated from multiple overwater dispersals from northern South America to the Lesser Antilles in the Plio-Pleistocene interval (3.2–1.4 mya), which included posterior reverse colonization from the Caribbean to mainland Central and South Americas (Baker and Genoways 1978; Stadelmann et al. 2007; Larsen et al. 2012; Novaes et al. 2021). This

pattern can also be observed in other insectivorous bats (e.g., Dávalos 2005, 2006; Genoways et al. 2005; Pavan et al. 2013). Dispersions may have been mediated by the migration arc formed by the expansion of land area of the Lesser Antilles islands due to sea level retreat during the Quaternary glaciations (Koopman 1958; Genoways

et al. 2010; Dávalos and Russell 2012; Allen et al. 2019; Hoffman et al. 2019). This was especially important for Grenada, Trinidad, Tobago, and northern South America, which allowed a great exchange of species (Koopman 1958; Genoways et al. 2010). Thus, the presence of *M. attenboroughi* in Grenada, Tobago, and Suriname – and probably along a broader area in northern South America – can be explained by the intermittent connections between these islands and the continent during the Plio-Pleistocene glaciations. In fact, some studies consider the fauna of Grenada and Grenadines (united as a single island during

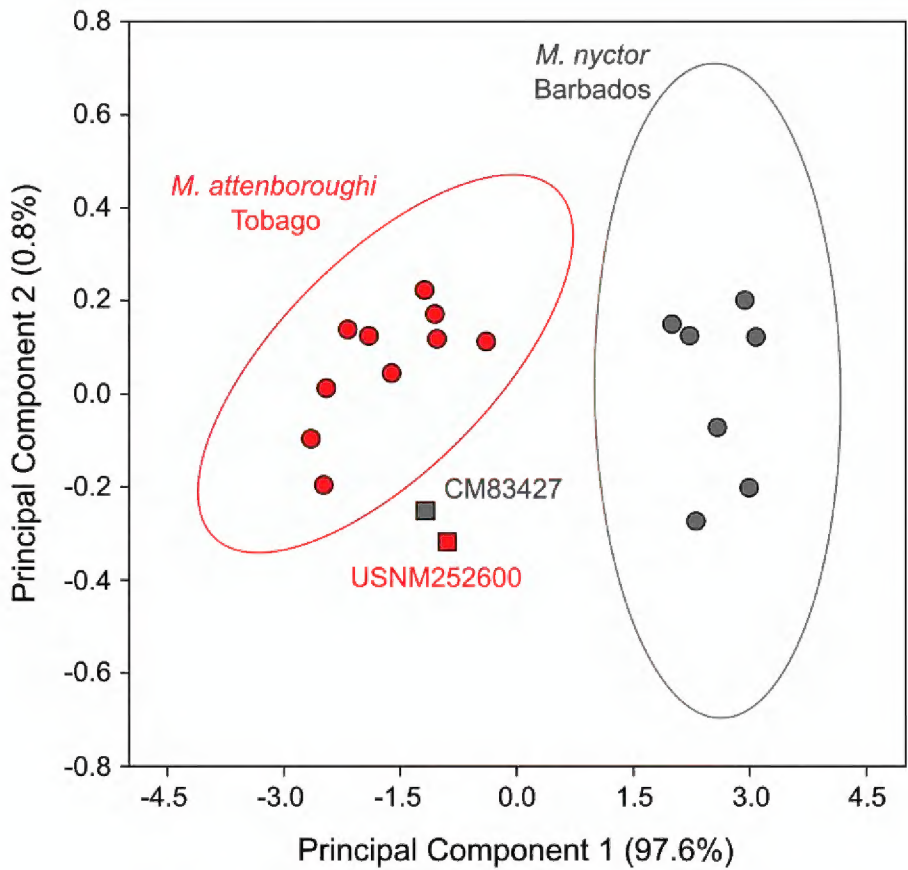


Figure 6. Dispersion points of the Principal Component Analysis based on skull measurements *Myotis attenboroughi* from Tobago (red dots), *Myotis nyctor* from Barbados (gray dots), the newly discovered *M. attenboroughi* from Grenada (red square; USNM 252600), and the specimen of *M. cf. nyctor* from Grenada (gray square; CM 83427).

Table 4. Vector correlation loadings with original variables of principal components analysis for *Myotis attenboroughi* and *M. nyctor* samples.

Measurements	PC 1	PC 2
MAL	0.331	0.260
MAN	0.194	0.243
GLS	0.369	-0.298
CCL	0.366	0.037
CBL	0.386	-0.178
CIL	0.408	-0.063
BAL	0.388	-0.143
MAB	0.168	-0.061
BCB	0.123	-0.163
IOB	0.032	0.650
POB	0.011	-0.157
BAC	0.130	0.215
BAM	0.124	0.317
MTL	0.182	0.242
M1M3	0.064	0.202

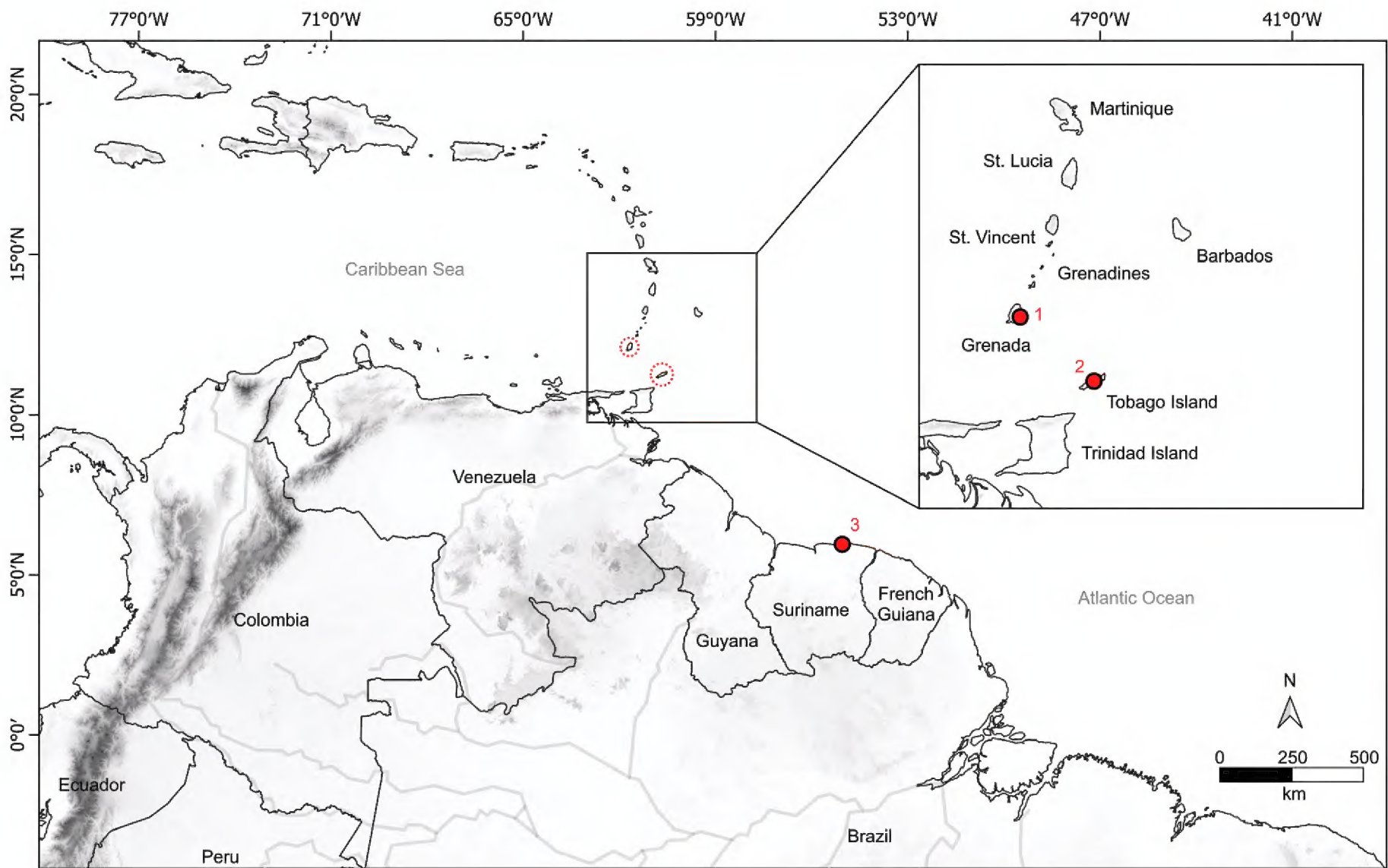


Figure 7. Occurrences localities of *Myotis attenboroughi* in Caribbean islands of Grenada (1) and Tobago (2); and mainland South America in Paramaribo, Suriname (3).

the Last Glacial Maximum) as small representation of the South American bat fauna (e.g., Koopman 1958; Genoways et al. 2010; Pavan et al. 2013).

On the other hand, the presence of a Grenadian *Myotis* genotypically closer to *M. nyctor* from Barbados cannot be explained by this scenario. Barbados and Grenada are separated by ca. 240 km in the Tobago Basin, with an ocean depth of more than 2,000 m and no oceanic ridges that may have facilitated the connection of these two populations during glaciation periods (Speed 1981; Humphrey 1997). In addition, Barbados was completely or partially underwater until 1 mya, and perhaps as recent as 700,000 years ago (Speed and Keller 1993; Lovette et al. 1999). So, the arrival of the *M. nyctor* ancestral lineage in Barbados is quite recent and probably resulted from an extreme overwater dispersal event from South America (Larsen et al. 2012a). Therefore, it is possible to assume that other similar events, perhaps driven by wind streams during tropical storms (Hurme et al. 2025), may have facilitated the invasion of *M. nyctor* from Barbados into Grenada. Subsequently, introgression of lineages may have occurred through hybridization, which can explain the discordance between the phenotype (like *M. attenboroughi*) and the genotype (like *M. nyctor*) of the specimen CM 83427 (JN020562) from Grenada. Recent studies reveal a complex evolutionary history of New World *Myotis*, which include several events of historical and modern introgression of lineages, hybridization, incomplete lineage sorting, and gene flow in phenotypically similar non-sister species (Morales and Carstens 2018; Platt et al. 2018; Korstian et al. 2022, 2024).

The hypotheses above are speculative, and new studies are necessary to understand the history of the Caribbean *Myotis* assemblage and the evolutionary processes linked to colonization and diversification. Genome-wide sequences for the Barbados and Grenada specimens should advance our ability to answer these questions since the cytb-only dataset seems to have limited resolution power for the Caribbean clade, restricting data interpretation (Novaes et al. 2021). Furthermore, recent studies have shown discordance between the mitochondrial and nuclear genomes of Neotropical *Myotis*, resulting from the aforementioned evolutionary phenomena (Platt et al. 2018; Korstian et al. 2024). Still, there is robust evidence indicating that the Caribbean is an important diversification center for bats, and that the fauna of the Lesser Antilles was formed from multiple overwater dispersal from northern South America, with reverse colonization of species that invaded the continent after speciation (Hedges et al. 1992; Dávalos 2004, 2007; Pavan et al. 2013; Rojas et al. 2016; Tavares et al. 2018). In this way, the high richness of species, endemism, and presence on the different islands makes *Myotis* an important model for research into biogeography and evolution of the Caribbean.

Author contributions

RLMN, VCC, and RM conceptualized the study; RLMN, VCC, EFA, DEW, JEM, and RM collected and generated

the data; RLMN and NAB analyzed the data; RLMN and VCC drafted the manuscript; all authors contributed to the theoretical conception of the study and contribute to the final version of the manuscript.

Data availability

All the data that support the findings of this study are available in the main text. DNA sequences generated in this study have been deposited in NCBI's GenBank. Morphological data matrices for all specimens are available upon request from the corresponding author.

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Appendix 1

Table A1. Cytochrome b sequences used in phylogenetic analyses. The information presented for the taxonomic terminals is the result of re-identification of the specimens and does not necessarily coincide with the original identifications provided by the authors and GenBank. Abbreviations for specimen deposit institutions are: Universidad Autónoma Metropolitana-Iztapalapa (UAMI, Ciudad de México, Mexico); Pontificia Universidad Católica del Ecuador (QCAZ, Quito, Ecuador), Carnegie Museum of Natural History (CM, Pittsburg, USA); Field Museum of Natural History (FMNH, Chicago, USA), Museum of Southwestern Biology, University of New Mexico (MSB, Albuquerque, USA), Museum of Vertebrate Zoology, University of California (MVZ, Berkeley, USA), University of Nebraska State Museum (UNSM-ZM, Lincoln, USA); Sam Noble Oklahoma Museum of Natural History (OMNH, Norman, USA); Smithsonian’s National Museum of Natural History (USNM, Washington, DC, USA); Texas Tech University (TTU, Lubbock, USA), Biology Department of Tunghai University (THUMB, Taichung, Taiwan). *The cytb sequence of *M. attenboroughi* from Grenada (USNM 252600) is available as Suppl. material 1.

Species	#GenBank	Voucher	Locality	Source
<i>Myotis albescens</i>	JX130463	TTU 85088	Pastaza, Ecuador	Larsen et al. (2012b)
<i>Myotis albescens</i>	JX130522	TTU 85091	Pastaza, Ecuador	Larsen et al. (2012b)
<i>Myotis albescens</i>	AF376839	FMNH 162543	Tarija, Bolivia	Ruedi and Mayer (2001)
<i>Myotis albescens</i>	JX130503	TTU 99124	Boquerón, Paraguay	Larsen et al. (2012b)
<i>Myotis albescens</i>	JX130504	TTU 99818	Ñeembucú, Paraguay	Larsen et al. (2012b)
<i>Myotis arescens</i>	OP270161	–	Araucanía, Chile	Novaes et al. (2022c)
<i>Myotis arescens</i>	OP270162	–	Araucanía, Chile	Novaes et al. (2022c)
<i>Myotis arescens</i>	OP270166	–	Coquimbo, Chile	Novaes et al. (2022c)
<i>Myotis arescens</i>	AM261888	–	Santiago, Chile	Stadelmann et al. (2007)
<i>Myotis attenboroughi</i>	JN020573	UNSM-ZM 29470	St. George Parish, Tobago	Larsen et al. (2012a)
<i>Myotis attenboroughi</i>	JN020574	UNSM-ZM 29483	St. George Parish, Tobago	Larsen et al. (2012a)
<i>Myotis attenboroughi</i>	PQ757917	USNM 540692	St. George Parish, Tobago	Present study
<i>Myotis attenboroughi</i> *	–	USNM 252600	St. David, Grenada Island	Present study
<i>Myotis attenboroughi</i>	JX130505	CM 77705	Paramaribo, Suriname	Larsen et al. (2012b)
<i>Myotis armiensis</i>	JX130435	TTU 39146	Chiriquí, Panama	Larsen et al. (2012b)
<i>Myotis armiensis</i>	MW025265	MSB 262089	Chiriquí, Panama	Carrión-Bonilla and Cook (2020)
<i>Myotis armiensis</i>	MW025266	MSB 262237	Chiriquí, Panamá	Carrión-Bonilla and Cook (2020)
<i>Myotis armiensis</i>	MW025267	MSB 262788	Chiriquí, Panamá	Carrión-Bonilla and Cook (2020)
<i>Myotis armiensis</i>	MW025268	MSB 262085	Chiriquí, Panamá	Carrión-Bonilla and Cook (2020)
<i>Myotis armiensis</i>	JX130514	TTU 85060	Tungurahua, Ecuador	Larsen et al. (2012b)
<i>Myotis armiensis</i>	MW025269	QCAZ 17245	Napo, Ecuador	Carrión-Bonilla and Cook (2020)
<i>Myotis armiensis</i>	MW025274	QCAZ 12461	Zamora Chinchipe, Ecuador	Carrión-Bonilla and Cook (2020)
<i>Myotis armiensis</i>	MZ345121	USNM 370890	Distrito Federal, Venezuela	Novaes et al. (2022a)
<i>Myotis atacamensis</i>	OP270158	–	Arica, Chile	Novaes et al. (2022c)
<i>Myotis atacamensis</i>	OP270159	–	Arica, Chile	Novaes et al. (2022c)
<i>Myotis atacamensis</i>	OP270160	–	Arica, Chile	Novaes et al. (2022c)
<i>Myotis bakeri</i>	AM261882	MVZ 168933	Olmos, Peru	Stadelmann et al. (2007)
<i>Myotis caucensis</i>	JX130484	CM 98860	Huánuco, Peru	Larsen et al. (2012b)
<i>Myotis caucensis</i>	JX130538	TTU 46346	Huánuco, Peru	Larsen et al. (2012b)
<i>Myotis causensis</i>	JX130495	QCAZ 6313	Pastaza, Ecuador	Larsen et al. (2012b)
<i>Myotis chiloensis</i>	OP270163	–	Los Lagos, Chile	Novaes et al. (2022c)
<i>Myotis chiloensis</i>	OP270164	–	Los Lagos, Chile	Novaes et al. (2022c)
<i>Myotis chiloensis</i>	OP270165	–	Los Lagos, Chile	Novaes et al. (2022c)
<i>Myotis clydejonesi</i>	JX130520	TTU 109227	Sipaliwini, Suriname	Larsen et al. (2012b)
<i>Myotis clydejonesi</i>	JX130453	CM 98859	Huánuco, Peru	Larsen et al. (2012b)
<i>Myotis diminutus</i>	JX130447	TTU 103805	Loja, Ecuador	Larsen et al. (2012b)
<i>Myotis diminutus</i>	JX130448	QCAZ 9601	Esmeraldas, Ecuador	Larsen et al. (2012b)
<i>Myotis diminutus</i>	JX130466	QCAZ 9154	Esmeraldas, Ecuador	Larsen et al. (2012b)
<i>Myotis diminutus</i>	JX130467	QCAZ 9155	Esmeraldas, Ecuador	Larsen et al. (2012b)
<i>Myotis dinellii</i>	JX130475	TTU 66489	Córdoba, Argentina	Larsen et al. (2012b)
<i>Myotis dinellii</i>	MT262853	MG-ZV-M 217	Zavalla, Argentina	Caraballo et al. (2020)
<i>Myotis dinellii</i>	MT262857	MG-ZV-M 233	Chanar Laneado, Argentina	Caraballo et al. (2020)
<i>Myotis dominicensis</i>	JN020555	TTU 31507	St. Joseph’s Parish, Dominica	Larsen et al. (2012a)
<i>Myotis dominicensis</i>	JN020556	TTU 31508	St. Joseph’s Parish, Dominica	Larsen et al. (2012a)
<i>Myotis dominicensis</i>	AF376848	–	St. Joseph’s Parish, Dominica	Ruedi and Mayer (2001)
<i>Myotis elegans</i>	JX130479	TTU 84380	Atlantida, Honduras	Larsen et al. (2012b)
<i>Myotis elegans</i>	JX130480	TTU 84138	Atlantida, Honduras	Larsen et al. (2012b)
<i>Myotis extremus</i>	AF376852	–	Yucatán, Mexico	Ruedi and Mayer (2001)

Species	#GenBank	Voucher	Locality	Source
<i>Myotis extremus</i>	JX130449	TTU 47514	Yucatán, Mexico	Larsen et al. (2012b)
<i>Myotis extremus</i>	JX130525	–	Yucatán, Mexico	Larsen et al. (2012b)
<i>Myotis extremus</i>	JX130489	CM 55764	Veracruz, Mexico	Larsen et al. (2012b)
<i>Myotis extremus</i>	MF143477	–	Veracruz, Mexico	Platt et al. (2018)
<i>Myotis extremus</i>	MW025270	MVZ 226977	Alta Verapaz, Guatemala	Carrión-Bonilla and Cook (2020)
<i>Myotis fortidens</i>	JX130437	–	Michoacán, Mexico	Larsen et al. (2012b)
<i>Myotis fortidens</i>	JX130439	–	Michoacán, Mexico	Larsen et al. (2012b)
<i>Myotis fortidens</i>	KC747690	LACM 73713	Guerrero, Mexico	Patrick and Stevens (2014)
<i>Myotis keaysi</i>	JX130516	QCAZ 11380	Chimborazo, Ecuador	Larsen et al. (2012b)
<i>Myotis keaysi</i>	JX130517	QCAZ 11383	Chimborazo, Ecuador	Larsen et al. (2012b)
<i>Myotis keaysi</i>	MW025273	MSB 70381	Cochabamba, Bolivia	Carrión-Bonilla and Cook (2020)
<i>Myotis larensis</i>	JN020569	TTU 48161	Guárico, Venezuela	Larsen et al. (2012b)
<i>Myotis larensis</i>	JX130529	TTU 48162	Guárico, Venezuela	Larsen et al. (2012b)
<i>Myotis larensis</i>	JX130535	CM 78645	Guárico, Venezuela	Larsen et al. (2012b)
<i>Myotis lavalii</i>	AF376864	MVZ 185681	Paraíba, Brazil	Ruedi and Mayer (2001)
<i>Myotis martiniquensis</i>	AM262332	–	Martinique Island	Stadelmann et al. (2007)
<i>Myotis martiniquensis</i>	JN020557	MNHN 2005-895	Grand'Rivière, Martinique	Larsen et al. (2012a)
<i>Myotis martiniquensis</i>	JN020558	MNHN 2005-896	Le Morne Rouge, Martinique	Larsen et al. (2012a)
<i>Myotis midastactus</i>	MW323450	USNM 584502	Santa Cruz, Bolivia	Novaes et al. (2022a)
<i>Myotis moratellii</i>	JX130572	QCAZ 9179	El Oro, Ecuador	Larsen et al. (2012b)
<i>Myotis moratellii</i>	MZ345120	USNM 513482	Los Ríos, Ecuador	Novaes et al. (2022a)
<i>Myotis nesopolus</i>	JN020575	–	Bonaire, Netherlands Antilles	Larsen et al. (2012a)
<i>Myotis nesopolus</i>	JN020576	–	Bonaire, Netherlands Antilles	Larsen et al. (2012a)
<i>Myotis nesopolus</i>	JN020577	–	Bonaire, Netherlands Antilles	Larsen et al. (2012a)
<i>Myotis nigricans</i>	OR187561	FMA 630	Rio de Janeiro, Brazil	Novaes et al. (2024)
<i>Myotis nigricans</i>	OR187562	FMA 957	Rio de Janeiro, Brazil	Novaes et al. (2024)
<i>Myotis nigricans</i>	PP584498	FMA 1525	Rio de Janeiro, Brazil	Novaes et al. (2024)
<i>Myotis nigricans</i>	PP584499	FMA 1534	Rio de Janeiro, Brazil	Novaes et al. (2024)
<i>Myotis</i> cf. <i>nyctor</i>	JN020562	CM 83427	St. David Parish, Grenada	Larsen et al. (2012a)
<i>Myotis nyctor</i>	JN020563	TTU 109225	St. Thomas Parish, Barbados	Larsen et al. (2012a)
<i>Myotis nyctor</i>	JN020564	TTU 109226	St. Thomas Parish, Barbados	Larsen et al. (2012a)
<i>Myotis nyctor</i>	JN020565	TTU 109229	St. Thomas Parish, Barbados	Larsen et al. (2012a)
<i>Myotis nyctor</i>	JN020566	TTU 109224	St. Thomas Parish, Barbados	Larsen et al. (2012a)
<i>Myotis nyctor</i>	JN020567	TTU 109230	St. Thomas Parish, Barbados	Larsen et al. (2012a)
<i>Myotis oxyotus</i>	JX130509	–	Loja, Ecuador	Larsen et al. (2012b)
<i>Myotis oxyotus</i>	JX130585	–	Loja, Ecuador	Larsen et al. (2012b)
<i>Myotis oxyotus</i>	MW089499	QCAZ 11739	Imbabura, Ecuador	Carrión-Bonilla et al. (2024)
<i>Myotis pilosatibialis</i>	JX130526	TTU 35360	San Luis Potosí, Mexico	Larsen et al. (2012b)
<i>Myotis pilosatibialis</i>	JX130518	TTU 35631	San Luis Potosí, Mexico	Larsen et al. (2012b)
<i>Myotis pilosatibialis</i>	MW025271	MVZ 226976	Alta Verapaz, Guatemala	Carrión-Bonilla and Cook (2020)
<i>Myotis pilosatibialis</i>	MW025272	MVZ 226973	El Quiche, Guatemala	Carrión-Bonilla and Cook (2020)
<i>Myotis pilosatibialis</i>	MW025275	MVZ 224798	Quezaltenango, Guatemala	Carrión-Bonilla and Cook (2020)
<i>Myotis pilosatibialis</i>	JX130519	TTU 60981	Santa Ana, El Salvador	Larsen et al. (2012b)
<i>Myotis riparius</i>	JX130492	TTU 102883	Esmeraldas, Ecuador	Larsen et al. (2012b)
<i>Myotis riparius</i>	JX130473	CM 68443	Para, Suriname	Larsen et al. (2012b)
<i>Myotis riparius</i>	JX130474	CM 78659	Bolívar, Venezuela	Larsen et al. (2012b)
<i>Myotis riparius</i>	MW089495	MSB 70383	Cochabamba, Bolivia	Carrión-Bonilla et al. (2024)
<i>Myotis riparius</i>	JX130485	TTU 99645	Paraguari, Paraguay	Larsen et al. (2012b)
<i>Myotis riparius</i>	MW089493	OMNH 36220	Tucumán, Argentina	Carrión-Bonilla et al. (2024)
<i>Myotis ruber</i>	AF376867	MVZ 185999	São Paulo, Brazil	Ruedi and Mayer (2001)
<i>Myotis simus</i>	JX130481	TTU 46348	Huánuco, Peru	Larsen et al. (2012b)
<i>Myotis velifer</i>	AF376870	MVZ 146766	Sonora, Mexico	Ruedi and Mayer (2001)
<i>Myotis velifer</i>	JX130438	UAMI 15306	Michoacán, Mexico	Larsen et al. (2012b)
<i>Myotis velifer</i>	JX130589	UAMI 15305	Michoacán, Mexico	Larsen et al. (2012b)
<i>Myotis yumanensis</i>	AF376875	MVZ 15585	California, USA	Stadelmann et al. (2007)
<i>Myotis</i> sp. 1	JN020570	CM 63933	Nickerie, Suriname	Larsen et al. (2012a)
<i>Myotis</i> sp. 1	JN020571	CM 69053	Para, Suriname	Larsen et al. (2012a)
<i>Myotis</i> sp. 1	JN020572	CM 77699	Para, Suriname	Larsen et al. (2012a)
<i>Myotis</i> sp. 1	JX130476	CM 77692	Marowjine, Suriname	Larsen et al. (2012b)
<i>Myotis</i> sp. 1	JX130534	CM 77694	Nickerie, Suriname	Larsen et al. (2012b)
<i>Myotis</i> sp. 1	JX130536	CM 77700	Para, Suriname	Larsen et al. (2012b)

Species	#GenBank	Voucher	Locality	Source
<i>Myotis</i> sp. 2	AF376865	FMNH 129208	Lima, Peru	Ruedi and Mayer (2001)
<i>Myotis</i> sp. 3	MT262866	MFA-ZV 1425	Esperanza, Argentina	Caraballo et al. (2020)
<i>Myotis</i> sp. 3	JX130450	TTU 34952	Puerto Linares, Bolívia	Larsen et al. (2012b)
<i>Myotis</i> sp. 3	JX130528	TTU 34953	Puerto Linares, Bolívia	Larsen et al. (2012b)
<i>Myotis</i> sp. 3	PP584500	UFMT 4946	Mato Grosso, Brazil	Novaes et al. (in press)
<i>Myotis</i> sp. 3	PP584501	MZUFV 5180	Mato Grosso, Brazil	Novaes et al. (in press)
<i>Myotis</i> sp. 3	JX130498	TTU 99046	Alto Paraguai, Paraguay	Larsen et al. (2012b)
<i>Myotis</i> sp. 3	JX130455	TTU 95992	Alto Paraguai, Paraguay	Larsen et al. (2012b)
<i>Myotis</i> sp. 3	JX130540	TTU 99151	Boquerón, Paraguay	Larsen et al. (2012b)
<i>Myotis</i> sp. 3	JX130539	TTU 99516	Concepción, Paraguay	Larsen et al. (2012b)
<i>Myotis</i> sp. 3	JX130499	TTU 99802	Ñeembucu, Paraguay	Larsen et al. (2012b)
<i>Myotis</i> sp. 3	JX130496	TTU 99743	Presidente Hayes, Paraguay	Larsen et al. (2012b)
Outgroups				
<i>Myotis emarginatus</i>	MK799667	FMNH 178892	Ajlun, Jordan	Patterson et al. (2019)
<i>Submyotodon latirostris</i>	KP187906	THUMB 30036	Heping, Taiwan	Ruedi et al. (2015)
<i>Kerivoula papillosa</i>	MG194454	FMNH 205343	Luzon I, Philippine Island	Sedlock et al. (2020)

Appendix 2

Specimens examined in morphological comparisons. These vouchers consist of fluid preserved specimens, stuffed skins, and skulls deposited in the American Museum of Natural History (AMNH, New York, United States); Carnegie Museum of Natural History (CM, Pittsburgh, United States); National Museum of Natural History, Smithsonian Institution (USNM, Washington, D.C., United States); Natural History Museum, University of Kansas (KU, Lawrence, United States).

Myotis attenboroughi (N = 14): Trinidad and Tobago: Tobago Island, Charlottesville, 1 km N of Pirate’s Bay, Saint John Parish (USNM 540693 [holotype], 540692 [paratype]); Tobago Island, St. Mary Parish, Hillsborough Reservoir (USNM 538064, 538065, 538066, 538067, 538068, 538069, 540619, 540620, 540621, 540694, 540695 [paratypes]). Grenada: St. David (USNM 252600).

Myotis nyctor (N = 8): Barbados: St. Thomas Parish, Cole’s cave (KU 151761, 151762, 151763, 151764, 151765, 151766, 109473 [paratype]); St. Thomas, Near Cole’s cave (AMNH 213926).

Myotis cf. *nyctor* (N = 1): Grenada, St. David (CM 83427).

Supplementary material 1

Supplementary data

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Data type: fasta

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